UNIVERSITY OF CALIFORNIA, AQUATIC TOXICOLOGY LABORATORY

QUALITY ASSURANCE PROJECT PLAN (UCD-ATL QAPP)

FOR

FIELD ACTIVITIES AND LABORATORY ANALYSES FOR BIOLOGICAL ASSESSMENT

(INTERIM DRAFT)

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University of California - Davis Project Manager (Tom Kimball)	Date
Contractor Contract Manager	Date

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1. GENERAL INTRODUCTION AND OBJECTIVES OF UCD-ATL QUALITY ASSURANCE PROJECT PLAN DOCUMENTS

The University of California, Davis - Aquatic Toxicology Laboratory (UCD-ATL) is a State Certified Laboratory whose primary purpose is to conduct toxicity tests to evaluate the water quality of water samples. In general, USEPA three-species toxicity testing methods and Toxicity Identification and Evaluation methods as defined by USEPA (1994) are used to characterize and identify potential contaminants in a sample. Other EPA and non-EPA tests also are conducted at the facility. The quality of the data generated at the UCD-ATL is ensured through a variety of protocol and criteria established by the USEPA and/or the UCD-ATL and implemented in the Laboratory. These include, but are not limited to, documentation of standard operating procedures, documentation of deviations from established protocol, as well as implementation of preventive and corrective measures to meet quality assurance objectives.

This Quality Assurance Project Plan (QAPP) document defines procedures and criteria that will be used in projects conducted by the UCD-ATL in association with a contracting individual, group or agency. Among other things, criteria for data quality acceptability, procedures for sampling, testing (including deviations) and calibration, as well as preventive and corrective measures are included in this document. The responsibilities of the UCD-ATL and of the contracting agency also are outlined. The methods and quality assurance protocols contained in this document are primarily those described in the UCD-ATL's Aquatic Bioassessment Standard Operating Procedures (2001).

A general description of the components of the project being contracted to the UCD-ATL is included in Section 2. Agencies, groups or individuals with whom UCD-ATL contracts with will be responsible for submitting a project description that includes a project overview and its goals as well as for submitting the site list and rationale, and sampling frequency to the UCD-ATL Project Manager. That group or individual also is responsible for determining the sampling sites, amounts of samples to be collected, types and number of tests to be conducted, and for designating sample collector(s).

QA project plans must be drafted and then approved by laboratory management prior to project initiation.

2. PROJECT BACKGROUND AND DESCRIPTION

The Contractor is responsible for submitting to the UCD-ATL Director a project description. It should include project objectives, a project overview and the rationale for the project. In addition, the Contractor must also submit a site list with map, site selection justification and sample collection frequency to the UCD-ATL Laboratory Manager prior to the initiation of the project. If known, the dates of sample collection should also be included.

2a. Methods

The protocol used will follow methods defined by UCD-ATL in their Aquatic Bioassessment Standard Operating Procedures (2001).

2b. Sample Collection

A scientific collecting permit(s), issued by CDFG's License and Revenue Branch (916-227-2225), must be obtained prior to any sample collection. On the permit application, it should be specified that freshwater invertebrates, incidental fish and amphibians (authorizations 5, 6 and 8, respectively) will be taken. Not every member of the sampling team needs to be in possession of this permit; however, the person whose name appears on the permit should be present and in possession of the permit during sample collection.

Samples will be collected by the Contractor or UCD-ATL according to CDFG's CSBP method. The non-point source sampling design will be used. In general, a family of riffles within a reach of a stream will be considered a sampling unit. Procedural modifications for low gradient streams and for narrow streams will be used when necessary. The sites and sampling times are described below in Section 2c.

2c. Sites and Sampling Times

Site reconnaissance will be conducted by UCD-ATL staff and are determined by the Contractor or after the Contractor consults with the UCD-ATL staff. Sampling frequencies depend on the purpose of the project.

2d. Physical/Habitat Quality Measurement

The scoring criteria for physical/habitat quality is based on USEPA nationally standardized methods.

2e. Sample Processing, Taxonomic Identification and Metric Analyses

The sample processing schedule will depend on collection frequency. Subsampling, taxonomic identification and metric analysis will be conducted in collaboration with trained CDFG personnel. All three BMI samples collected per reach will be processed.

2f. Water Quality Task

Monthly trends in water quality monitoring will be conducted. Specific water quality measurements are listed below:

- Temperature Specific Conductivity
- Dissolved Oxygen
- pH
- Turbidity
- Photo Documentation

Listed below are water quality parameters that will be measured in addition to the monthly tends in water quality monitoring. The additional water quality measurements will be conducted on a site-specific basis.

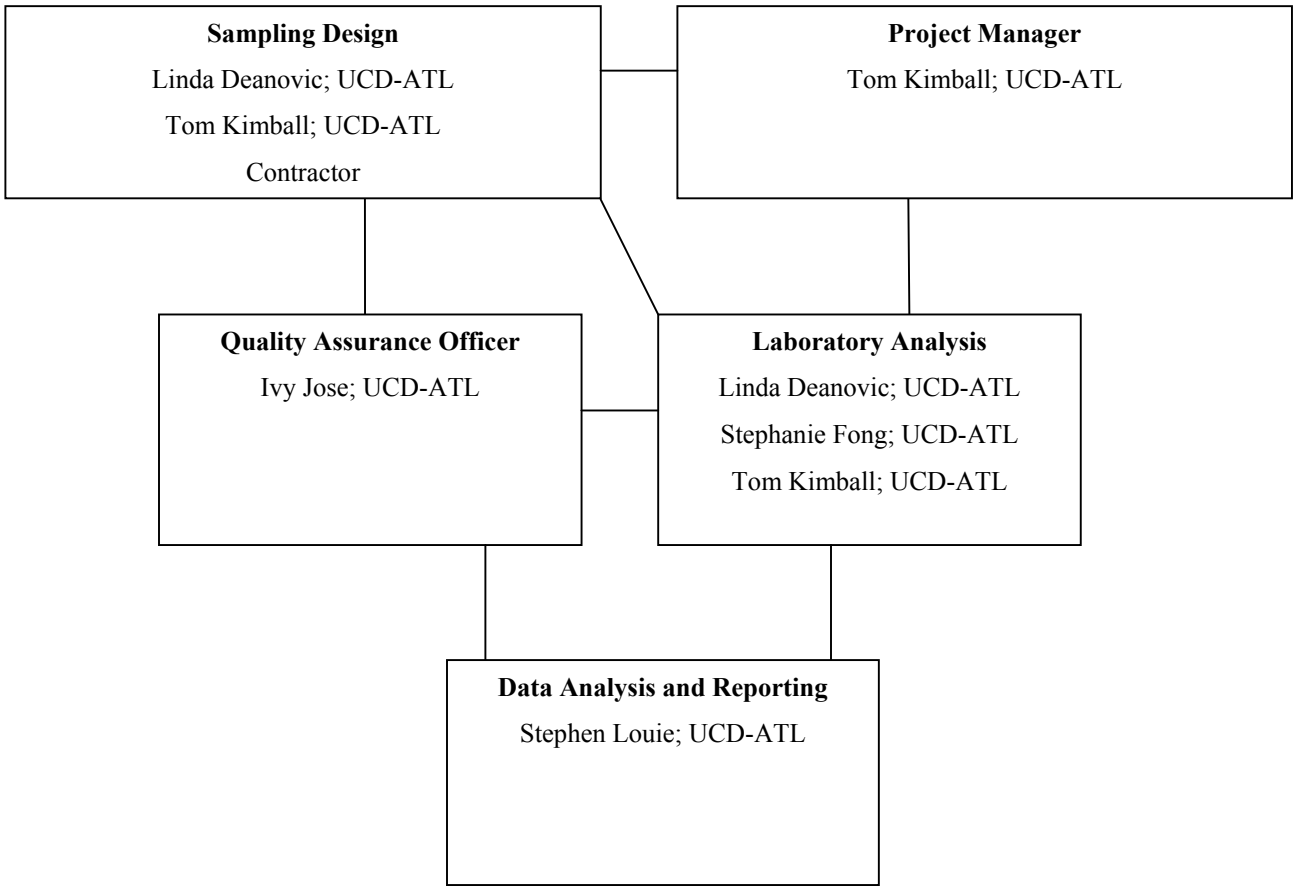
- Hardness
- Alkalinity
- Mean Velocity/Depth
- BOD
- TOC
- Nitrite, Nitrate and TKN
- Ortho-phosphate and Phosphate
- TSS
- Ammonia-Nitrogen

3. PROJECT ORGANIZATION AND RESPONSIBILTY

All UCD-ATL staff involved in this project will be trained on field and laboratory procedures. Additional training will be provided over the course of the project. The Project Manager, Tom Kimball, has had the most experience and formal training in most aspects of the bioassessment procedures.

RESPONSIBILITIES	PERSON
Sampling:	
Sampling Design/Site Reconnaissance	Linda Deanovic; UC Davis Tom Kimball;UC Davis Contractor
Sample collection, shipment, field analysis; Physical/ Habitat Measurements	Tom Kimball; UC Davis
Calibration of field instruments	Tom Kimball, laboratory technicians; UC Davis
Sample Storage	Tom Kimball; UC Davis
Sample Processing	Linda Deanovic; UC Davis Tom Kimball; UC Davis
Taxonomic Identification and Metric Analysis	Linda Deanovic; UC Davis Tom Kimball; UC Davis
Sample Processing Quality Control, data validation, audits, and corrective action	Melenee Emanuel; UC Davis
Project Direction	Dr. Charles Plopper; UC Davis
Contract Management	Contractor
Project Quality Assurance	Ivy Jose; UC Davis
Statistical Guidance	Neil Willits; UC Davis
Data Management and Reporting	Stephen Louie; UC Davis

Figure 1. Summary diagram of lines of communication.



4. FIELD PROCEDURES

Because this will be the first bioassessment study to be conducted by the UCD-ATL, field procedures still have to be written for inclusion into the laboratory's SOP manual. Since the procedures already have been standardized by CDFG, the UCD-ATL will follow CDFG procedures. Once the project is underway, any deviations from this protocol will be documented and reported by the Project Manager to the QA Officer and the Contract Manager. Any procedural deviations also will be documented in the reports. The procedure that follows was taken directly from the CDFG protocol (1999) and from procedural modifications described by other sources (CCRWQCB, 1999; Harrington and Born, 1999-2000).

4a. Field Equipment and Supplies

The following field equipment/supplies will be needed:

- Measuring tape (100 m)
- D-shaped kick net (0.5 mm mesh)
- Standard Size 35 sieve (0.5 mm mesh)
- Wide-mouth 500 ml plastic jars
- White sorting pan and forceps
- 95% ethanol
- pH, temperature, DO and conductivity meter
- Stadia rod and hand level/clinometer
- Densiometer/Solar Pathfinder
- GPS unit or watershed topographic map
- Velocity meter
- Digital camera

The following forms should also be with the samplers in the field (a copy of each of these forms are attached at the end of this plan). "Write in the rain" paper will be used for the forms.

- California Bioassessment Worksheet (CBW)
- Physical/Habitat Quality Form: Standard Form and Form for Low-Gradient Streams
- Chain of Custody Form
- Random Number Table
- Bioassessment Sample Labels (includes the following information: Riffle/Reach Number, Transect Number, Stream Name, Date/Time, Sampler Name)

4b. Non-Point Source Sampling Design for Use When Riffle Habitats are Present

This sampling design that follows will be used when riffle habitats are available. The sampling units are riffles within a reach of stream. At least five or more riffles must be present within the same stream order and relative gradient. The samplers should collect one BMI sample from the upstream third of five randomly chosen riffles.

1) Randomly choose 3 riffles within the stream reach using the random number table.
2) Starting with the downstream riffle, place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Use a random number table to select 1 transect from all possible meter marks along the top third of the riffle.
3) Inspect the transect before collecting BMI samples by imagining a line going from one bank to the other, perpendicular to the flow. Choose 3 locations along that line where you will place your net to

collect BMIs. If the substrate is fairly similar and there is no structure along the transect, the 3 locations will include the side margins and the center of the stream. If there is substrate and structure complexity along the transect, then as much as possible, select the 3 collections to reflect it.

4) After mentally locating the 3 areas, collect BMIs by placing the D-shaped kick-net on the substrate and disturbing a 1x2 foot portion of substrate upstream of the kick-net to approximately 4 to 6 inches in depth. Pick-up and scrub large rocks by hand under water in front of the net. Maintain a consistent sampling effort (approximately 1 to 3 minutes) at each site. Combine the three collections within the kick-net to make one composite sample. Apply the same sampling effort when collecting each sample. Some substrate types may require more time than others but the effort would be similar.

5) Place the contents of the kick-net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove the water before placing the material in the jar. According to King (Bioassessment Services), this step is optional and not recommended since sifting the benthos sample in a sieve may damage organisms. An alternative would be to rinse sample to cod end of net and carefully transfer to the sample container. A pan is placed under the container to catch any sample spilled during transfer.

6) The sampled material should be stored in a jar with 95% ethanol. Do not fill a jar more than 2/3 full with sampled material and gently agitate jars that contain primarily mud or sand. A label with all the following information should be placed in each jar: Riffle/Reach Number, Transect Number, Stream Name, Date/Time, Sampler Name(s).

7) Proceeding upstream, Repeat Steps 2 through 6 for the next two riffles within the stream reach.

4c. Field Procedure Modification for Sampling Narrow Streams

In cases where the stream's width is too narrow to accommodate the collection of five net-widths across, randomly pick three 1x2 square foot areas from all the possible 1x2 sampleable areas in the riffle. This concept is presented more clearly in Figures 8-1 below (Harrington and Born 1999-2000).

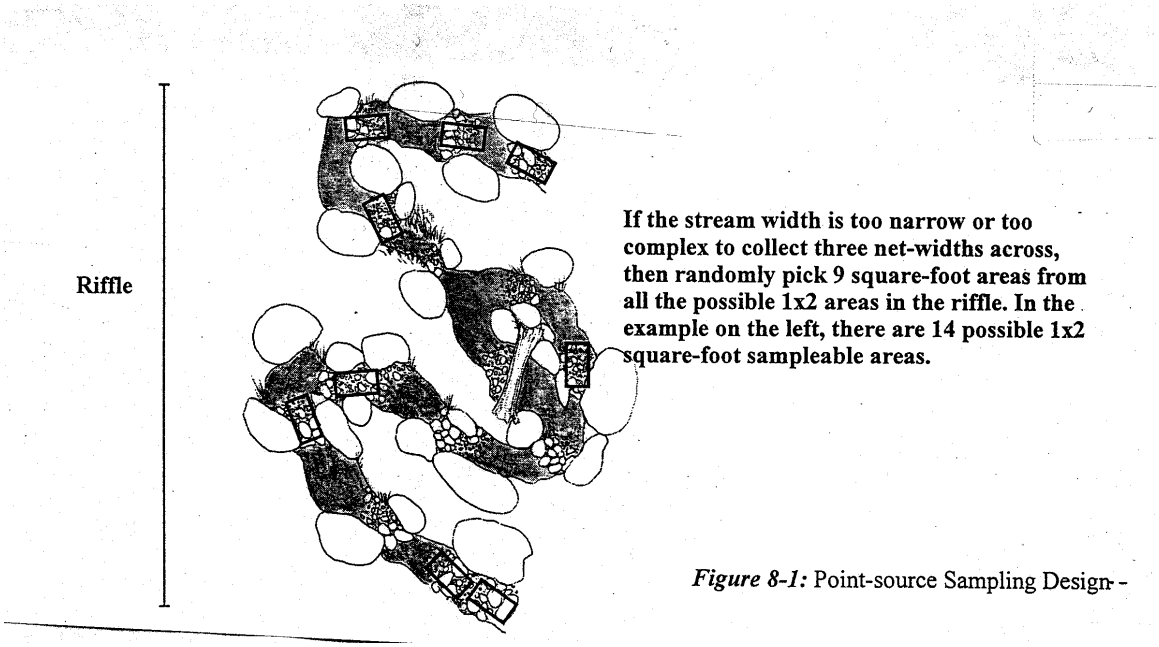


Figure 8-1: Point-source Sampling Design -

4d. Field Procedure Modifications for Sampling Streams with Sand and Mud Bottoms

The following procedures are applicable when sampling streams with sand or mud bottoms. These procedures have been adapted from the Central Coast Regional Water Quality Control Board/Salinas Watershed Aquatic Bioassessment Protocol Brief (1999) and are modeled after CSBP and USEPA Rapid Bioassessment Protocol. Revisions by J. T. King of Bioassessment Services also are incorporated. The habitat assessment field data sheet for low gradient streams should be used in this case.

1) Select a 100 meter reach representative of the characteristics of the stream in the area of interest. In order to minimize structural effects on velocity, depth and overall habitat quality, the area chosen should be located at least 100 meters upstream from any road or bridge crossing. There should be no major tributaries discharging into the stream in the study area.

2) Stretch a 100 m measuring tape along the bank. Each meter mark represents a possible transect location. Using a random number table, randomly select three samples from all possible meter marks along the meter tape.

3) Beginning at the downstream most transect, sampling proceeds at an angle perpendicular to stream flow. Collect samples at the side margins and the stream thalweg along each transect.

4) Place the D-frame kick net on the substrate. Ensure that flow is moving through the net to carry debris into it. Disturb a 1X2 foot portion of substrate upstream of the net to approximately 4 to 6 inches in depth. Physically scrub all rocks by hand under water in front of the net. Disturb the substrate for 60 seconds or more at each site. Sixty seconds may be reasonable if all the benthos is sand, however, other substrate types may take longer. Combine the three samples from each transect to make one composite sample.

5) Sift the contents of the net by first placing it in a standard size 35 sieve (0.5 mm mesh) and removing large twigs, leaves and rocks by hand after careful inspection and retrieval of clinging organisms. According to King (Bioassessment Services), this step is optional and not recommended since sifting the benthos sample in a sieve may damage organisms. An alternative would be to rinse sample to cod end of net and carefully transfer to the sample container. Place a pan under the container to catch any sample spilled during transfer.

6) The sampled material should be stored in a jar with 95% ethanol. Do not fill a jar more than 2/3 full with sampled material and gently agitate jars that contain primarily mud or sand. A label with all the following information should be placed in each jar: Riffle/Reach Number, Transect Number, Stream Name, Date/Time, Sampler Name(s).

7) Proceeding upstream, Repeat Steps 3 through 6 for the next two randomly chosen riffles.

4e. Measurement of Physical/Habitat Quality

The physical/habitat scoring criteria is used to measure the physical integrity of a stream and is a USEPA nationally standardized method. CDFG recommends that this procedure be conducted on every reach of stream sampled as part of a bioassessment program. The Physical/Habitat Quality Form should be filled out for the entire reach where the BMI samples were collected as part of the non-point source sampling design. A modified version is used for low gradient streams. This procedure is an effective measure of a stream's physical/habitat quality, but requires field training prior to using it and the implementation of quality assurance measures throughout the field season.

4f. Measurement of Chemical and Physical/Habitat Characteristics

Measurements of the chemical and physical/habitat characteristics are used to describe the riffle environment and helps in the interpretation of BMI data. The information can be used to classify stream reaches and to explain anomalies that might occur in the data but are not necessarily a good substitute for a quantitative fisheries habitat survey.

The following procedures will be used to measure chemical and physical/habitat characteristics:

1) Water temperature, specific conductance, pH and dissolved oxygen should be measured at the sampling site using approved standardized procedures and instruments. Collect a sample for turbidity measurement.

1) Record the riffle length determined for the procedure to choose the transect locations. Estimate the average riffle width or transect by averaging several measurements along its length. Measure the riffle depth by placing the stadia rod at several places within the riffle and averaging the measurements.

2) Estimate or measure the entire length of the reach where three to five riffles are chosen as part of the non-point source sampling design use in the low gradient transect method. A reach that contains five riffles will be optimal.

3) Measure the riffle velocity using a flow meter placed in front of the three locations along the transect(s) where the BMI samples were collected. Average the readings.

4) Estimate the percent of the riffle surface that is covered by shade from streamside vegetation (canopy cover) using a densiometer at several places along the riffle and averaging the readings.

5) Determine substrate complexity and embeddedness by applying Parameter 1 and 2, respectively from the Physical/Habitat Quality Form to the riffle where the BMI sample was collected. Use the entire riffle to assess these parameters and make note if the area along the transect (s) is considerably different from the rest of the riffle.

6) Visually estimate the percent of riffle in each of the following substrate categories: fines (<0.1"), gravel (0.1-2"), cobble (2-10"), boulder (>10") and bedrock (solid). Use the entire riffle to assess this parameter and make note if the area along the transect(s) is considerably different from the rest of the riffle.

7) Estimate substrate consolidation by kicking the substrate with the heel of your wader boots to note whether it is loosely, moderately or tightly cemented. The estimate should also take into consideration the hands-on experience obtained from collecting the BMI sample.

8) Measure the gradient or slope of the riffle using a stadia rod and hand level or a clinometer.

4g. California Bioassessment Worksheet

A California Bioassessment Worksheet (CBW) should be filled out for the entire reach when using the Non-point Design. Follow the procedure below for filling out the CBW.

1) Enter the watershed and stream name, date and time of sample collection, name of the company or agency collecting the samples, sample identification number(s) and a short description on the CBW.

2) Enter the names of each crew member in the Crew Member Box.

3) Determine the longtitude and latitude coordinates and elevation from a GPS unit or watershed topographic map. Determine which California ecoregion or sub-ecoregion the site is located in by using the U.S. Forest Service map. This map can be obtained from the California Aquatic Bioassessment Web site. Record this information and any other comments on the sampling site in the Site Location Box.

4) Record the water temperature, specific conductance, pH and dissolved oxygen measurements in the Chemical Characteristics Box. Collect a turbidity sample.

5) Record the physical/habitat characteristics in the Riffle/Reach Characteristics Box. For the non-point source sampling design, record the reach length, the total score from the Physical/Habitat Quality Form and all physical/habitat characteristics information on the lines below the "riffle 1 through riffle 3" columns.

6) Record the name and address of the Bioassessment Laboratory that received the samples along with the laboratory sample numbers if they are different than the field sample identification numbers.

4h. Sample Custody

A chain of custody (COC) form must be completed following the procedure below. The original will be retained at the UCD-ATL, and a copy will be retained by the agency project supervisor.

1) At the end of the field day, record the following information on the COC for each group of BMI samples: program name; watershed name, field ID numbers, sampling dates, and the name, address, telephone number and signature of one of the crew members collecting the sample.
2) Field samples and COCs must remain in a locked sample depository until a decision has been made to send them to a bioassessment laboratory for processing.
3) When transporting to the bioassessment laboratory, each group of samples must be accompanied by a COC. Upon delivery, a Bioassessment Laboratory Number will be assigned to each sample. Record this number on the COC and each individual CBW along with the name and address of the bioassessment laboratory. When all samples listed on the COC are accounted for, then the individual delivering the samples will sign the "Released By" portion and the laboratory personnel will signed the "Received By" portion of the COC. The original COC is to remain at the laboratory and a copy will be retained by the project supervisor.

5. LABORATORY PROCEDURES

The CSBP has three levels of BMI identification. The procedure described below corresponds to Level 3, the professional level equivalent and requires identification of BMIs to a standard level of taxonomy. Again, all procedures described below are taken directly from CDFG's protocol (1999). UCD-ATL-specific protocols have yet to be written. CDFG states that all professional bioassessment laboratories should belong to the California Bioassessment Laboratories Network (CAMLnet), an organization that provides technical assistance to laboratories and ensures that laboratory efforts are consistent throughout California. The UCD-ATL has contacted this group and when needed, will use the group as a resource over the course of this project.

5a. Laboratory Equipment and Supplies

The following laboratory equipment and supplies will be used:

- Dissecting microscopes
- Standard size 35 sieve (0.5 mm)
- Gridded picking tray
- Wide-mouth glass jars
- Glass petri dishes
- Vials
- Taxonomic Keys
- 70% EtOH/5% glycerol
- Fine dissection forceps
- Turbidimeter
- Standardized taxonomic list
- Waterproof paper/pencils
- Laboratory benchsheets
- Random number table generator
- Chain of custody form

5b. Sub-sampling methods

1) Retrieve the sample from the sample depository and crosscheck the sample number with the bioassessment laboratory number on the COC.

2) Empty the contents of the sample jar into the #35 sieve (0.5 mm mesh) and thoroughly rinse with water. Care must be taken when performing this step to minimize damage to organisms.

3) Once the sample is rinsed, clean and remove debris larger than 1/2 inch. Remove and discard green leaves, twigs and rocks. Do not remove filamentous algae and skeletonized leaves.

4) After cleaning, place the material into a plastic tray marked with equally sized, numbered grids (approximately 2x2 inches). Do not allow any excess water into the tray. Spread the moist, cleaned debris on the bottom of the tray using as many grids necessary to obtain an approximate thickness of 1/2 inch. Make an effort to distribute the material as evenly as possible.

5) Remove and count macroinvertebrates from randomly chosen grids until 300 BMIs are removed. Place the BMIs in a clean petri dish containing 70% ethanol/5% glycerin. Completely count the remaining organisms in the last grid but do not include them with the 300 used for identification. The final count should be recorded on the benchsheet for eventual abundance calculations.

6) The debris from processed grids should be put in a clean remnant jar and the remaining contents of the tray should be placed back into the original sample jar. Both jars should be filled with fresh 70% ethanol, labeled (bioassessment laboratory number and either "original" or "remnant") and returned to the sample depository.

5c. Taxonomic Identification

1) Identify the 300 BMIs from each sample to the standardized level recommended by CAMLnet using appropriate taxonomic keys.

2) Place identified BMIs in individual glass vials for each taxon. Each vial should contain a label with taxonomic name, bioassessment laboratory number, stream, county, collection date and collector's name. This voucher should be labeled and returned to the sample depository.

3) Record taxonomic information on a Macroinvertebrate Laboratory Bench Sheet. The bench sheet should include the following information: watershed or project name, sampling date, sample ID number, bioassessment laboratory number, date of subsampling, name of subsampler, remnant jar number, taxonomy completion date, name of taxonomist, taxonomic list of organisms and enumeration, total number of organisms, total number of taxa, list of unknowns, problem groups and comments.

4) Maintain a reference collection of representative specimens of all accurately identified BMI taxa.

6. QUALITY ACCEPTABILITY PROCEDURES

The UCD-ATL will follow CDFG QA for this project. These are described below and are taken directly from the CDFG (1999). The data will be considered acceptable if the following quality assurance measures for both field and laboratory procedures are taken.

6a. Field Quality Assurance: Collection of Benthic Macroinvertebrate Samples

The CSBP is designed to produce consistent, random samples of BMIs. It is important to prevent bias in riffle choice and transect placement. The following procedures should be used to help field crews collect unbiased and consistent BMI samples.

1) In using the CSBP, most sampling reaches should contain riffles that are at least 10 meters long, one meter wide and have a homogenous gravel/cobble substrate with swift water velocity. There are modifications of the CSBP when these conditions do not exist. Methods to sample narrow streams and wadeable streams with muddy and sandy bottoms also are described above.

2) All field crew members will receive training in the use of the BMI sampling procedures. Field personnel should review the CSBPs before each field season.

3) During the training, crew members should practice collecting BMI samples as described in the CSBP. The 2 sq ft area upstream of the sampling device should be delineated using the measuring tape or a metal grid and the collection effort should be timed. Practice repeatedly until each crew member has demonstrated sampling consistency.

4) Throughout the sampling season, assure that effort and sampling area remain consistent by timing sampling effort and measuring sampled area for approximately 20% of the sampling events. The results should be discussed immediately and need not be reported.

6b. Field Quality Assurance: Physical/Habitat Quality Measurement

Physical/habitat parameters are assessed based on visual evaluation and is inherently subjective. The rapid ranking system assesses parameters using a range from optimal to poor conditions. Although having experienced and consistent field crews is an advantage in sampling BMIs, it can be a problem with assessing physical/habitat quality. The subjective nature of this procedure can lead a field crew in a direction that is considerably different from the other field crews. The following QA/QC procedures should be used to standardize individual observations and reduce differences in scores.

1) A DFG biologist or project supervisor should train field crews in the use of the EPA physical/habitat assessment procedures. Detailed descriptions of these procedures can be procured from DFG or the California Aquatic Bioassessment Web site. Field personnel should review these procedures before each field season.

2) At the beginning of each field season, all crew members should conduct a physical/habitat assessment of two practice stream reaches. Assess the first stream reach as a team and discuss in detail each of the 10 physical/habitat parameters described in the EPA procedure. Assess the second stream reach individually and when members are finished, discuss the 10 parameters and resolve discrepancies.

3) Frequently change or alternate assessment responsibilities of field crew members. At the end of each field day, crew members should discuss habitat assessment results and resolve discrepancies.

4) The Project Supervisor should randomly pre-select 10 to 20% of the stream reaches where each crew member will be asked to assess the physical/habitat parameters separately. The discrepancies in individual crew member scores should be discussed and resolved with the Project Supervisor.

6c. Laboratory Quality Assurance

Laboratory analysis of macroinvertebrate samples can be a significant cost for bioassessment programs. The CSBP specifies identification of BMIs to a standard level of taxonomy, usually to genus and/or species level. The CSBP also requires sub-sampling procedures using a fixed count of 300 organisms. Employing these procedures with confidence requires an effective quality assurance program. Complete quality assurance compliance will require a minimal 10% cost overhead. However, it will allow for testing whether sub-sampling, organism enumeration and taxonomic identification are consistent and accurate. The following procedures will be used to ensure quality data is produced:

6c-1. The California Macroinvertebrate Laboratory Network (CAMLnet)

All individuals or groups using the CSBP laboratory procedures should get information on CAMLnet, a group consisting of personnel from bioassessment laboratories throughout California. The group provides a forum where laboratory procedures are discussed and the BMI taxonomic levels are determined. It also provides taxonomic workshops and assistance with interlaboratory taxonomic verification. The UCD-ATL has contacted the person in charge of this group (Peter Ode, CDFG). Currently, membership is voluntary, no fees are assessed and laboratories or members who join are placed on a mailing list. The UCD-ATL requested information on the group in September 2000. The UCD-ATL is now on the CAMLnet mailing list and will use the network as a technical resource when necessary.

6c-2. Standard Operating Procedures (SOPs)

1) The laboratory will include SOPs in the existing UCD-ATL SOP manual that outline bioassessment procedures as defined by CSBP, but with detailed instructions specific to the laboratory. Any procedural modifications also will be included in the SOP manual. These SOPs will be maintained and updated regularly. The assigned personnel and duties of the Laboratory Supervisor and QA Taxonomist should be specified in the SOP.

2) Customized benchsheets will be developed for each phase of subsampling and identification.

6c-3. Sample Handling and Custody

1) When samples arrive, laboratory staff should inspect the samples for sufficient volume of ethanol and the labels for pertinent information including waterbody, sample date and time, location, transect number and sampler name. The steps discussed in Section 4f should be followed.

2) The sample description information should be recorded in the Laboratory Sample Inventory Log and each sample given a unique identification number. A written and electronic record should be maintained to trace the samples from entry into the laboratory through final analysis. Samples should be stored in the sample repository until processing and returned there after processing.

6c-4. Sample Archiving and Storage

Sample archiving will consist of the following steps.

1) After the sample(s) is/are collected and water is removed, the sample material will be placed in a jar with 95% ethanol.

2) Within one week of sample collection, sample(s) integrity will be inspected and the storage ethanol will be replaced with fresh 95% ethanol.

3) A second and final 95% ethanol exchange will be conducted on the sample(s) three weeks after sample collection. The sample(s) will be stored up to one year in the final 95% ethanol replacement. If the sample is stored longer than one year, the 95% will be replaced to preserve sample integrity.

Sample(s) will be stored in a flammable cabinet at less than or equal to 25ºC, until sample disposal has been approved.

6c-5. Laboratory Crew Training

1) All laboratory crew must be trained on the use of the CSBP before working in the laboratory.

2) The crew will review the laboratory procedures before analysis begins. This should be done regardless of the laboratory's previous taxonomic experience. Taxonomic identification and enumeration will only be conducted by laboratory crew members after proper training. Samples for identification will be stored in preservative to prevent sample deterioration.

6c-6. Subsampling Accuracy

Sub-sampling involves removing 300 organisms for each sample, or all organisms if the entire sample contains less than 300. The procedure to estimate abundance usually requires removing more than 300 organisms for each sample; however, only 300 are retained for identification. The sub-sampling technician systematically transfers organisms from the sample to a collection vial then transfers the processed sample debris into a Remnant jar. The QA taxonomist should examine at least 10% of the Remnant samples for overlooked organisms. For sub-samples containing 300 or more organisms, the Remnant sample should contain fewer than 10% of the total organisms sub-sampled. The Remnant for samples containing fewer than 300 organisms should contain fewer than 30 organisms.

6c-7. Taxonomic Identification and Enumeration

The CSBP requires that all organisms are identified to a standardized taxonomic level using established taxonomic keys and references. The QA taxonomist should check at least 10% of the samples for taxonomic accuracy and enumeration of individuals within each taxon. The same sample numbers that were selected randomly for the sub-

sampling quality control should be used for this procedure. Misidentification and/or taxonomic discrepancies as well as enumeration errors should be noted in the laboratory bench sheets. The Laboratory Supervisor determines if the errors warrant corrective action.

6c-8. Organism Recovery

1) During the sorting and identification process, organisms may be lost, miscounted or discarded. Taxonomists will record the number of organisms discarded and a justification for discarding on the laboratory bench sheets. Organisms may be discarded for several reasons including
   a) sub-sampler mistakes (e.g. inclusion of terrestrial or semi-aquatic organisms or exuviae,
   b) small size (<0.5mm)
   c) poor condition
   d) fragments of organisms.
2) The number of organisms recovered at the end of the sample processing will also be recorded and a percent recovery determined for all samples. Concern is warranted when organism recoveries fall below 90%. Samples with recoveries below 90% should be checked for counting errors and laboratory bench sheets should be checked to determine the number of discarded organisms. If the number of discarded organisms is high, then the technician that performed the sub-sampling should be informed and re-trained if necessary.

7. INTERLABORATORY TAXONOMIC VALIDATION

An external laboratory or taxonomic specialist should be consulted on a regular basis to verify taxonomic accuracy. External validation can be performed on selected taxa to help laboratory taxonomists with problem groups of BMIs and to verify representative specimens of all taxa assembled in a reference collection.

8. BIOASSESSMENT VALIDATION

The CSBP recommends that at least 10% bioassessment validation where whole samples of 300 identified BMIs are randomly selected from all samples either for a particular project of for all samples processed within a set time period, such as each 6 months or a year. The labels should be removed from the vials and replaced with coded labels that do not show the taxonomic names of the BMIs. The validation laboratory or specialist should be instructed to identify and enumerate all specimens in each vial and produce a taxonomic list. There will inevitably be disagreements between the bioassessment and the external laboratory on taxonomic identification. These taxa should be re-examined by both parties and a resolution reached before a final QA report is written. CDFG is still currently working on this QA technique to determine the acceptable level of misidentification and appropriate corrective actions.

9. CORRECTIVE ACTION

Any quality control parameter that is considered out of range should be followed by a standard corrective action that includes two levels. Level 1 corrective action includes an investigation for the source of error or discrepancy derived from the quality control parameter. Level II corrective action includes checking all samples for the error derived from the quality control parameter, but is initiated only after the results of the Level I process justify it. The decision to initiate Level II corrective action and reanalyze samples or conduct quality control on additional samples should be made by the laboratory supervisor.

10. DATA DEVELOPMENT AND ANALYSIS OF BIOASSESSMENT RESULTS

The CSBP analysis procedures are based on the EPA's multi-metric approach to bioassessment data analysis. The EPA is developing procedures for multi-variate analysis of bioassessment data. The sampling protocols were designed to facilitate multi-variate analysis.

A taxonomic list of the BMIs identified for each sample should be generated for each project along with a table of sample values and means for the biological metrics required. Variability of the sample values should be expressed

as the coefficient of variability (CV). Significance testing can be used for point source sampling and ranking procedures can be used to compare sites sampled using the non-point source design. Ultimately, there will be a regional Index of Biological Integrity to compare sample site mean values.

In accordance with the CVRWQCB contract (Holmes, 2000), the following taxonomic lists and functional feeding positions, and bioassessment metrics shall be generated: taxa richness, EPT taxa, EPT index, Dipteran taxa, non-insect taxa, sensitive EPT index, Shannon Diversity Index, tolerance value, percent dominant taxa, percent Hydropsychidae, percent Baetidae, percent Diptera, percent non-insects, percent Chironomidae, percent intolerant organisms, percent tolerant organisms, percent collectors, percent filterers, percent grazers, percent predators, percent shredders, modified Hilsenhoff Family Biotic Index, percent dominant taxon, relative abundance and other metrics as deemed appropriate. Procedures for calculating most of the above biological metrics are described in Section 10 of Harrington and Born's manual (1999).

11. LABORATORY INSTRUMENT CALIBRATION PROCEDURES AND FREQUENCY

Laboratory instruments will be calibrated, standardized or maintained according to procedures detailed in the Standard Operating Procedures Manual (SOP manual). New SOPs will need to be written for new field and laboratory equipment. For each instrument, the procedures identify step-by-step calibration and maintenance procedures, corrective action (troubleshooting procedures), and record keeping. The conductivity and pH meters will be checked against known standards every six weeks for precision. Thermometer temperatures will be checked against a NIST certified calibrating thermometer annually to determine the degree error. The temperature correction will be identified on the thermometers. Data generated from the quality assurance checks will be implemented into a control chart.

Field instruments will be calibrated and their calibration recorded in the field logbook prior to use. These procedures are present in the SOP manual as well (see Part 12, pages 23 - 24).

12. PREVENTIVE MAINTENANCE OF FIELD AND LABORATORY EQUIPMENT

Laboratory and field instruments will be calibrated, standardized or maintained according to procedures detailed in the Standard Operating Procedures Manual (UCD-ATL, 1995). Each SOP contains instructions for: step-by-step calibration and maintenance procedures, corrective action (troubleshooting procedures), and record keeping. Until laboratory-specific SOPs can be written for certain field equipment to be used in this project, the manufacturer's instructions will be used.

13. PERFORMANCE AND SYSTEM AUDIT

The Contract Manager will conduct quarterly inspections of the physical facilities, operational systems, and operating procedures in the facility. The inspections will be conducted while sample processing are being performed. The facility will be given 24-hour notice of the inspections. In addition to the Contract Managers' inspection, the Quality Assurance Officer will conduct systems audits biannually.

The quarterly quality assurance reports will be approved by the laboratory QA Officer and evaluated by the Contract Manager. Deviations from the procedures outlined in this QA Project Plan will be brought to the attention of the Contract Manager. Corrective action will be taken to address all concerns.

Performance audits will be conducted biannually by the Quality Assurance Officer to assure that all procedures outlined in the UCD-ATL SOPs are followed by the laboratory staff.

14. CORRECTIVE ACTION, DEVIATIONS AND PROTOCOL AMENDMENTS

Corrective action may be taken if the quality assurance objectives outlined previously are not met. The Contract Manager will be informed, either by the Project Manager, QA Officer or a designated member of the UCD-ATL Staff, of any corrective action or deviation from the SOPs and the QAPP. These also will be documented and reported in the quarterly and final reports.

15. DATA REDUCTION, VALIDATION, AND REPORTING (SEE DATA DEVELOPMENT AND ANALYSIS PAGE)

According to CDFG's CSBP protocol brief, all groups and individuals conducting bioassessment in California will receive an Access database program for the purposes of data storage, processing and data-sharing. The UCD-ATL is in the process of acquiring this and will use the program in keeping with standardizing data for comparison and presentation with other laboratories conducting bioassessment project.

All statistical and mathematical computations will be double-checked.

Data will be double-checked for transcription errors after it is entered into the computer.

The University's QA officer will double-check and investigate discrepancies between field and laboratory measurements of water chemistry. Field and laboratory measurements are expected to be variable due to transport time and temperature changes. A significant discrepancy is defined as a difference greater than 1.0 pH units and an electrical conductivity having greater than 20% variability. Significant discrepancies will be noted and reported in the final report.

When UCD-ATL is unable to resolve an issue that arises in the laboratory, a literature search and /or expertise in the field will be consulted.

16. QUALITY ASSURANCE REPORTS TO MANAGEMENT

The Project Director will produce quarterly progress reports that include quality assurance information. These reports will describe the work performed to date, the results of completed processing, problems encountered, and an assessment of the effect of these problems on test results and a description of measures taken to correct problems. The following schedule will be followed by the UCD-ATL.

Items	Timeline schedule
Generated QAPP	
Monitoring Plan	
Sampling	
First Quarterly Report	30 days after the 1st Quarter of CSBP
Second Quarterly Report	30 days after the 2nd Quarter of CSBP
Third Quarterly Report	30 days after the 3rd Quarter of CSBP
Fourth Quarterly Report	30 days after the 4th Quarter of CSBP
Draft Final Draft	3 months after the 4th Quarter
Review Period	3 to 4 months after the 4th Quarter
Final Report	7 months after the 4th Quarter

17. LITERATURE CITED

CCRWQCB (Central Coast Regional Water Quality Control Board). 1999. Salinas River Watershed Bioassessment Procedure: protocol brief for biological and physical/habitat assessment in wadeable streams. With revisions by J. Thomas King, Bioassessment Services, June 2000.

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Harrington, J. and M. Born. 1999-2000. Measuring the health of California streams and rivers. A methods manual for: water resource professionals, citizen monitors and natural resources students. Second Edition. © Sustainable Land Stewardship International Institute. Sacramento, CA.

Holmes, R. 2000. Draft of Request for Contract with UC Davis. Draft as of 18 August 2000. Central Valley Regional Water Quality Control Board, Sacramento River Watershed Unit. Sacramento, CA. (in prep)

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USEPA (United States Environmental Protection Agency). 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Third Edition. Lewis, P.A., D.J. Klemm, *et al*. (eds.) *EPA/600/4-91/002*. Environmental Monitoring Systems Laboratory, Office of Research and Development. US Environmental Protection Agency. Cincinnati, OH.

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18. ATTACHMENTS

For reference purposes, the following forms are attached:

In most cases, the forms attached have been adapted from CDFG versions.

1) A map with the sites marked (from CVRWQCB Contract Manager)
2) Copy of California Bioassessment Worksheet
3) Physical/Habitat Quality Form and Modified form for Low Gradient Streams
4) Chain of Custody Form
5) Random Number Table
6) Bioassessment Sample Labels